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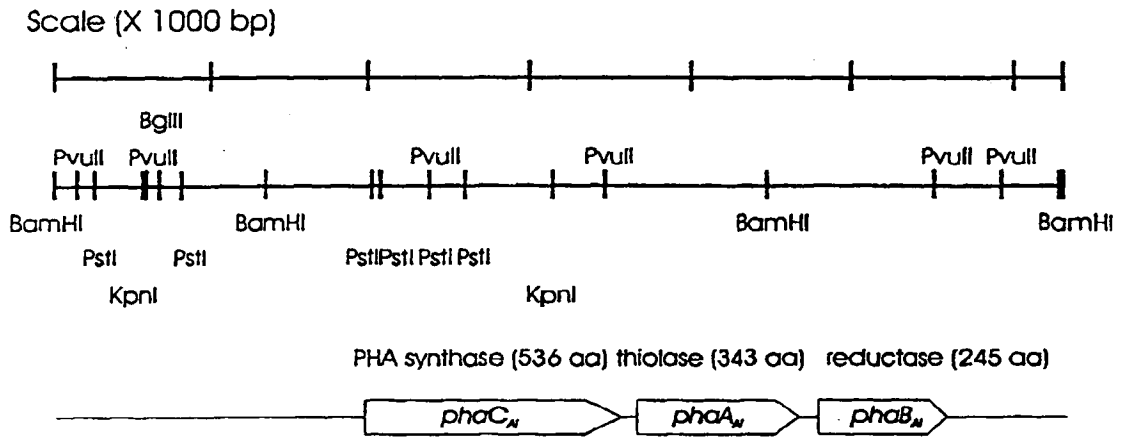

(57) Abstract

There is diclosed a PHA biosynthesis-related DNA fragment, which comprises the genes for PHA synthase, β-ketothiolase and acetoacetyl-CoA reductase, which are all derived from *Alcaligenes latus*. The DNA fragment is inserted in an expression vector. *E. coli* which is transformed with the expression vector carrying the DNA fragment can produce the PHA biosynthesis-related enzymes as well as accumulate PHA at a large quantity by culturing it in one-step.

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POLYHYDROXYALKANOATE BIOSYNTHESIS-RELATED GENES DERIVED FROM *Alcaligenes latus*

BACKGROUND OF THE INVENTION

Field of the invention

The present invention relates to polyhydroxyalkanoate (hereinafter referred to as "PHA") biosynthesis-related genes for PHA synthase, β-ketothiolase and acetoacetyl-CoA reductase, derived from *Alcaligenes latus*, their amino acid sequences, a recombinant plasmid carrying these genes, and a method for massproducing PHA using these gene. Also, the present invention relates to polyhydroxybutyrate(hereinafter referred to as "PHB") gene derived from *Alcaligenes latus*, its amino acid sequence and a recombinant plasmid carrying PHB gene, and a method for mass-producing PHB using the gene.

Description of the Prior Art

Petroleum synthetic plastics are so durable that they are not degraded in usual conditions at all. Because the production amount of the petroleum synthetic plastics increases each year, the environmental pollution ascribed to petroleum synthetic plastics wastes are now a big social problem. To solve the problem of non-degradable plastics, active research and development efforts have been and continued to be directed to biodegradable polymers all over the world.

Biodegradable polymers are the high molecular weight materials that are completely degraded under natural conditions after a period of time. Many biodegradable polymers have been developed. Of them, PHA, a natural polyester which is synthesized and accumulated by microorganisms, is of particular interest because it is superior in biodegradability as well as shows

physical properties similar to those of the synthetic plastics in current use (Anderson A.J. and Dawes, E.A., *Microbiol. Rev.*, 1990, 54, 450-472; Lee, S.Y., *Biotechnol. Bioeng.*, 49:1-14,1996; Lee, S.Y., *Trends Biotechnol.*, 14:431-438, 1996).

In detail, PHA is an organic reserve material, which can provide an intracellular store of carbon or energy, usually found in *Pseudomonas*, *Alcaligenes*, *Azotobacter*, and *Bacillus* spp.,etc. It is detectable as granular cytoplasmic inclusions. As a general rule, the cellular content of the reserve material is relatively low in actively growing cells: They accumulate massively when cells are limited in nitrogen, phosphorous, sulfur, oxygen, etc., but still have carbon and energy available. This reserve material was first found in *Bacillus megaterium* by Lemoigne in 1925 (Lemoigne, M., *Bull. Soc. Chem. Biol.*, 8:770-782, 1926). Since then, its chemical and physical properties have been extensively researched. Poly(3-hydroxybutyrate) is the most widely and first known PHA.

According to the number of carbon atoms and the substituents in hydroxyalkanoate, many PHAs were reported. In general, PHAs are divided into two classes ; short-chain-length PHAs(SCL PHAs) and medium-chain-length PHAs(MCL PHAs)

SCL PHAs include poly-β-hydroxypropionic acid, poly-β-hydroxybutyric acid, and poly-β-hydroxyvaleric acid, which are produced by *Alcaligenes eutrophus*, *Azotobacter vinelandii*, *methylotrophs,* etc. SCL PHAs are widely used due to their similar properties to polypropylene, a kind of chemically synthesized plastics.

MCL PHAs, composed of 3 to 9 more carbon atoms than SCL PHAs, are produced by *Pseudomonas* spp., by using alkane, 1-alkene, $C_6 \sim C_{12}$ alkanoic acids as a carbon.

Since early the 1960s, it was recognized that PHA could work like thermoplastic polymers. Thereafter, attracting a great attention, many types of PHA copolymers were synthesized, which are superior in mechanical properties as well as in biodegradability. By virtue of these advantages and owing to the environmental pollution aggravated by petroleum synthetic polymer wastes, PHA is now actively researched and developed as an alternative for plastics over the world. In addition, biocompatibility and bioabsorptivity allow PHA to be used in a variety of fields, as materials for agriculture, medicinal care, drug transfer system, and package, and as precursors for fine chemical products (Holmes, P.A. in Developments in crystalline polymers. 1-65, 1988).

Taking advantage of various bacteria, molecular biological research has revealed that there are four different biosynthetic pathway for PHA (Steinbuchel, A. in Biomaterials: novel materials from biological sources, 215-262, 1991). For example, for *Alcaligenes eutrophus*, the most widely known bacteria, β-ketothiolase, acetoacetyl-CoA reductase and polyhydroxyalkanoate synthase (PHA synthase) are known to be involved in the biosynthesis of PHA (People, O.P. and Shinskey, A.J., *J. Biol. Chem.*, 264: 15298-15303, 1989; Schubert, P., Steinbuchel, A. and Schlegel, H.G., *J. Bacteriol.*, 170:5837-5847, 1988; Slater, S.C., Voige, W.H. and Dennis, D.E., *J. Bacteriol.*, 170:4431-4436, 1988).

A concrete biosynthetic pathway of PHA in *Alcaligenes eutrophus*, gram negative bacteria, is as follows. Between two molecules of acetyl-CoA, a carbon-carbon bond forms in the presence of β-ketothiolase, the product of gene *phbA* , according to a biological Claisen condensation. The acetoacetyl-CoA thus formed is converted into D(-)-β-hydroxybutyryl-CoA by the stereoselective reduction of NADPH-dependent acetoacetyl-CoA reductase, the

product of gene *phbB*. Finally, D(-)-β-hydroxybutyryl-CoA is polymerized via ester bond by PHA synthase, the product of gene *phbC*.

In order to clone the genes which pertain to the biosynthesis of PHA in other bacteria than *Alcaligenes* eutrophus, much effort has been made. That is, the comprehension of the biosynthesis of PHA in bacteria makes it possible efficient production of PHA, versatility of substrates, synthesis of new PHA, and development of biopolymers similar to PHA. Further, recombinant strains which are obtained by utilizing the PHA biosynthesis-related genes can synthesize various PHAs at high efficiencies, resulting in a scientific and industrial significance (Lee, S.Y., *Trends Biotechnol.*, 14:431-438, 1996).

Strain *Alcaligenes latus* is reported to be so superior in the production of PHA that it accumulates PHA in cells at a proportion of around 90%. Also, *Alcaligenes latus* has the advantage in that it grows fast and uses inexpensive substrates as carbon sources (Wang, F. and Lee, S.Y., *Appl. Environ. Microbiol.*, 63:3703-3706, 1997). Unlike *Alcaligenes eutrophus*, *Alcaligenes latus* accumulates PHAs while they are growing. Thus, *Alcaligenes latus* can mass-produce PHA by one-step culture although the amount is low relative to that upon *Alcaligenes eutrophus*.

The use of *Alcaligenes latus* to produce PHA began in earnest in the mid-1980s by Chemie Linz AG, Austria. Biotechnologishe forchungesellschaft mbH, Austria, developed a process in which a one-step culture of strain btF-96, a mutant strain of *Alcaligenes latus*., produces PHA, asserting that one ton of PHA is obtained from a 15 m^3 fermentor per week (Hrabak, O., *FEMS Microbiol. Rev.*, 103:251-256, 1992). *Alcaligenes latus* also produces poly(3-hydroxybutyrate/3-hydroxypropionate) as well as poly(3-hydroxybutyrate/4-hydroxypropionate) in a medium containing disaccharides as carbon source by addition of 3-hydroxypropionate and γ-butyrolactone (Hiramitsu, M., Koyama, N., and Doi, Y., *Biotechnol. Lett.*, 15:461-464, 1993).

PHA can be produced by chemical process as well as biological process. However, Commercially favorable production scale of PHA is possible only by biological process. Since the production cost of PHA is much higher than those of other commercially available synthetic polymers, new technologies are required to reduce the production cost of PHA. Particularly, recombinant DNA technology gives a great contribution to the development and modification of novel strains, showing the production of novel polymers, utility of low-priced substrate, high efficiency of production, and facility in separation and purification. In order to develop such recombinant strains, first of all, it is necessary to understand the enzymes involved in the biosynthetic pathway for PHA.

In order to mass-produce biodegradable, natural PHA and its copolymers, the inventors have cloned genes for polyhydroxyalkanoate synthase, β-ketothiolase, and acetoacetyl–CoA reductase, and determined amino acid sequences and gene sequences. They have made expression vectors carrying the above genes and transformants, whereby polyhydroxyalkanoate can be produced and accumulated.

In addition, the inventors have cloned gene for polyhydroxybutyrate (PHB) and determined gene sequence and amino acid sequence, and made expression vector carrying the PHB gene and transformant, whereby polyhydroxybutyrate can be produced and accumulated.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a photograph showing opaque colonies of recombinant *E. coli* containing PHA biosynthesis-related genes derived from *Alcaligenes latus*, formed on a solid medium.

Fig. 2 is a photograph showing that recombinant *E. coli* containing PHA biosynthesis-related genes accumulates PHA in a broth.

Fig. 3 is a base sequence 6.4 kb in size, which contains the whole PHA biosynthesis-related genes derived from *Alcaligenes latus*.

Fig. 4 shows a restriction enzyme map of a 6.4 kb DNA fragment containing PHA biosynthesis-related genes derived from *Alcaligenes latus*, along with a gene structure.

Fig. 5 shows the gene structure of recombinant expression vector pJC1 carrying PHA biosynthesis-related genes derived from *Alcaligenes latus*.

Fig. 6 shows the process of preparing the recombinant expression vector carrying PHB synthase gene derived from *Alcaligenes latus*.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a polyhydroxyalkanoate biosynthesis-related gene.

The present invention provides an expression vector containing the polyhydroxyalkanoate biosynthesis- related gene and its transformant.

The present invention provides the method of preparing the polyhydroxyalkanoate synthase.

The present invention provides the method of preparing the polyhydroxyalkanoate.

In addition, the present invention provides a polyhydroxybutyrate gene.

The present invention provides an expression vector containing the polyhydroxybutyrate gene and its transformant.

The present invention provides the method of preparing the polyhydroxybutyrate synthase.

The present invention provides the method of preparing the polyhydroxybutyrate.

In the present invention, genes for the biosynthesis of PHA, are separated from *Alcaligenes latus*, which accumulates PHA while growing, whereby biodegradable, natural and industrially useful PHA and its copolymers can be mass-produced.

In more detail, the total genomic DNA separated from *Alcaligenes latus* is partly digested by restriction enzymes and the resulting DNA fragments are inserted into vector pUC19. *E. coli* is transformed with vector pUC19, followed by the selection of the recombinant vectors with a PHA biosynthesis-related DNA. The bacteria harboring the interest DNA was observed to accumulate PHA on a solid medium and in a liquid medium, as shown in Figs. 1 and 2, respectively.

Isolation of the recombinant vector from the transformed bacteria capable of producing PHA, is the first thing necessary to identify the DNA fragment of interest. Various analytic works show that the DNA fragment of interest is 6.4 kb in size, containing the genes coding for all of the β-ketothiolase, acetoacetyl-CoA reductase and PHA synthase.

Therefore, in accordance with an aspect, the present invention pertains to a PHA biosynthesis-related DNA fragment containing a PHA synthase gene, a β-ketothiolase gene and an acetoacetyl-CoA reductase gene, in due order, which has a size of 1608 bp (corresponding to 536 aa), 1176 bp (392 aa) and 735 bp (245 aa), respectively (see, Fig. 4).

Sequencing analyses reveal that the PHA synthase gene has a base sequence of Sequence 2 with a corresponding amino acid sequence of Sequence 5, as suggested in the accompanying Sequence Lists. The β-ketothiolase gene has a base sequence of Sequence 3 and the β-ketothiolase expressed therefrom

has an amino acid sequence of Sequence 6. The analyses also give that the acetoacetyl-CoA reductase gene has a base sequence of Sequence 4 which corresponds to an amino acid sequence of Sequence 7(see, Fig. 3 and Sequence Lists).

The recombinant vector anchoring the DNA for biosynthesis of PHA was named pJC1 (see, Fig. 5) and the transformant, *E. coli* XL-1 Blue/pJC1, was deposited in Korean Collection for Type Cultures, Korean Research Institute of Bioscience and Biotechnology on Nov. 5, 1997 and received a Deposition No. KCTC 0398 BP.

In accordance with another aspect, the present invention pertains to the preparation of the PHA biosynthesis-related enzymes by culturing host bacteria which harbor a recombinant expression vector containing the PHA biosynthesis-related genes.

In accordance with a further aspect, the present invention pertains to the production of PHA and its copolymers by use of the above host bacteria which can express the PHA biosynthesis-related genes. To this end, *E. coli* was transformed by the recombinant expression vector and after selecting, the transformed *E. coli* was cultured in a liquid medium containing glucose in suitable concentration to produce PHA. Where the *E. coli* was cultured in this manner, PHA was observed to accumulate until it represent as much as 40 % or more of the dry cell weight.

In addition, this invention provides polyhydroxybutyrate synthase (hereinafter referred to as "PHB synthase") and genes thereof. The total genomic DNA separated from *Alcaligenes latus* is partly digested by restriction enzyme, followed by selecting the DNA fragment showing positive signal by use of PHB gene derived from *Alcaligenes eutrophus* H16 as a probe. Plasmid vector pAL32 is obtained by inserting the above PHB gene into pSK(+).

The pAL32 is digested with *Eco*RI and *Not*I to obtain the PHB gene and then the resulting gene is inserted into plasmid pK230 of broad host range to obtain the recombinant expression vector pKTC32. This pKTC32 can express the gene in various host cells.(see Fig. 6)

The transformant *Alcaligenes eutrophus* LAR5 obtained by inserting pKTC32 into *Alcaligenes eutrophus* DSM541 which is lacking in PHB gene, was deposited in Korean Collection for Type Cultures, Korean Research institute of Bioscience and Biotechnology on Nov. 11, 1997, with a deposition No. KCTC 0568 BP.

When the above transformant *Alcaligenes eutrophus* DSM541(phb$^-$) /pKTC32 is cultured , it is observed that PHB synthase is produced in the cell cytoplasm in the form of white particle.

The invention will now be illustrated by the following examples, but not be limited in scope by reason of any of the following examples.

EXAMPLE I : Separation of Genomic DNA from *Alcaligenes latus*

The strain *Alcaligenes latus* (Wang, F and Lee, S.Y., *Appl. Envirn. Microbiol.*, 63:3707-3706, 1997) was cultured overnight in 500 ml of an NB medium (8 g/L nutrient broth). The bacteria in an initial stage of exponential growth were harvested by centrifugation and washed twice with saline-EDTA (0.15 M NaCl, 0.1 M EDTA, pH 8.0). The washed bacteria were suspended in 40 ml of 0.1 M saline-Tris-Cl (0.1 M NaCl, 10 mM EDTA, pH 9.0) and 1 ml of lysozyme solution (20 mg/ml) prepared just before use was added to the suspension. This suspension was dropwise added at 37 ℃ with Tris-SDS buffer (0.4 M NaCl, 1 mM EDTA, 20 mM Tris-Cl, pH 7.5, added with 5% SDS) with slow agitation. When the resulting solution became viscous, 5.5 ml of Proteinase K (10 mg/ml) was added and the total solution was incubated at

37 ℃ for 2 hours to remove proteins. Next, equal volume of phenol was added to the solution and well mixed for 30 min at room temperature with caution. After the solution was centrifuged at 6,000 rpm for 10 min, the supernatant was transferred to a fresh beaker followed by volume-measurement, and slowly added with two times the volume of cold ethanol to precipitate the genomic DNA which was, then, rolled up with a glass bar. The DNA was dried at room temperature and dissolved in 10 ml of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). Thereafter RNase was added to the above solution until the final concentration became 50μg/ml and the total solution was incubated at 37 ℃ for 1 hour. Then the same following process, i.e. mixing with phenol, centrifugation, volume mearsurement, addition of cold ethanol, rolling up, drying, and resuspension in TE buffer, was repeated. The only difference was that the concentration of TE buffer was 2ml.

EXAMPLE II : Cloning of PHA Biosynthesis-Related Genes

The genomic DNA of *Alcaligenes latus*, obtained Example I, was partly digested by restriction enzyme *Sau*3AI. Because restriction enzyme *Sau3*AI recognizes a specific four-base sequence in double-stranded DNA and cleaves both strands of the duplex at a specific site, various DNA fragments ranging from a small size to a large size can be obtained. These DNA fragments were separated according to size by electrophoresis on a low-melting temperature agarose gel.

To obtain the whole PHA biosynthesis-related gene, only the genes which were as large as or larger than 4 kb in size, were selected and inserted in plasmid pUC19 2.68 kb in size. To this end, first, the plasmid was cut with restriction enzyme *Bam*HI which leaves the same end sequence with restriction enzyme *Sau3*AI. Then, the genomic DNA fragments at least 4 kb long were

ligated with the opened plasmid vector pUC19 by using T4 DNA ligase (New England Biolabs).

The recombinant vector thus obtained was used to transform *E. coli* XL1-Blue (Stratagene) with the aid of an electroporator. When the recombinant vector pUC19 which contained the whole PHA biosynthesis-related gene at a *Bam*HI cloning site was taken up by *E. coli* XL1-Blue, white colonies were formed on a solid LB medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L) supplemented with ampicillin, X-gal (5-bromo-4-chloro-3-indolyl-β--D-galactopyranoside) and IPTG (isopropyl-1-thio-β--D-galactopyranoside). On the other hand, where the bacteria contained plasmid vector pUC19 without a DNA insert, blue colonies were formed. Through this procedure, colonies containing plasmid vector pUC19 with a partial genomic DNA insert of *Alcaligenes latus*, were selected. In order to determine whether these colonies were able to produce PHA, they each were inoculated in a broth capable of accumulating PHA.

In result, recombinant *E. coli* which was able to accumulate PHA, was obtained. From the recombinant *E. coli*, the recombinant plasmid vector was separated. An analysis data showed that the recombinant plasmid vector pUC19 anchored a partial genomic DNA of *Alcaligenes latus*, 6.4 kb long and that this DNA fragment contained the PHA synthesis-related genes. In addition, base sequencing analysis revealed that the 6.4 kb DNA fragment coded for all of the PHA biosynthesis-related enzymes, that is, β-ketothiolase, acetoacetyl-CoA reductase and PHA synthase.

In the present invention, the recombinant expression vector was named pJC1. The transformant which harbored plasmid pJC1 was deposited in Korean Collection for Type Cultures, Korean Research Institute of Bioscience and Biotechnology on Nov. 5, 1997, with a deposition No. KCTC 0398 BP.

EXAMPLE III : Structure Analysis of PHA Genes Derived from *A. latus*

The 6.4 kb DNA insert ligated to the plasmid vector pUC19 was analyzed to contain all the genes for ß-ketothiolase, acetoacetyl-CoA reductase and PHA synthase. These genes were positioned in the order of PHA synthase, ß-ketothiolase and acetoacetyl-CoA reductase from the 5' end to the 3' end.

Regarding the sizes of the PHA biosynthesis genes, the PHA synthase gene, β-ketothiolase gene and acetoacetyl-CoA reductase gene were 1608 bp (536 aa), 1176 bp (392 aa) and 735 bp (245 aa) long, respectively.

EXAMPLE IV : PHA-Producing Recombinant *E. coli* Containing PHA Biosynthesis-Related Genes Derived from *A. latus*

The recombinant expression vector pJC1 anchoring the 6.4 kb genomic DNA fragment of *Alcaligenes latus* was used to transform *E. coli* XL1-Blue. Since the bacteria which took up the recombinant expression vector could grow in a medium containing ampicillin, selection of the *E. coli* transformants was made on a solid medium containing 100 g/ml ampicillin. The selected *E. coli* was cultured in a defined or complex liquid medium containing 20 g/l glucose to produce PHA. When the strain was cultured at a temperature of 30 or 37 ℃ in a flask, PHA was accumulated until it represented as much as 40 % or more of the dry cell weight.

As described hereinbefore, the PHA biosynthesis-related genes of the present invention are derived from *Alcaligenes latus* and contains all of the genes for PHA synthase, ß-ketothiolase and acetoacetyl-CoA reductase. When *E. coli* is transformed with the PHA biosynthesis-related genes of the present invention, a one-step culture of the transformant *E. coli* can mass-produce

PHA. In addition, these enzymes and the genes are very helpful in understanding the biosynthesis of PHA in a molecular biological level.

The present invention has been described in an illustrative manner, and it is to be understood the terminology used is intended to be in the nature of description rather than of limitation.

Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it is to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

EXAMPLE V : Separation of PHB gene from *Alcaligenes latus* and determination of its DNA and amino acid sequence

In order to separate PHB gene, total DNA extracted from culture of *Alcaligenes latus* and digested with restriction enzymes such as *Bam*HI, *Hind*III, *Sma*I, *Xho*I, and *Sal*I and the DNA fragment was obtained.

Among the resulting DNA fragments digested with *Bam*HI, the 3.2 kb DNA showing positive signal , was separated by using 1 kb PHB gene derived from *Alcaligenes eutrophus* as a probe.

Then the separated DNA was ligated to the *Bam*HI restriction site of the vector pSK(+), whereby recombinant plasmid pAL32 was constructed. (see Fig. 5)

As the result of analyzing the pAL32 DNA sequence by Sanger Method (dideoxy-nucleotide chain termination method), it has revealed that the PHB gene derived from Alcaligenes latus consists of 1,608 bp. The amino acid sequence of the PHB synthase encoded by the above PHB gene, was analyzed

by using PC/Gene software program. PHB synthase derived from *Alcaligenes latus* has the amino acid sequence composed by 536 amino acids.

EXAMPLE VI : Construction of recombinant expression vector pKTC32 containing PHB gene

PHB gene is obtained by digesting pAL32 with *EcoR*I and *Not*I, and then the resulting DNA fragment was ligated to the restriction site by *EcoR*I and *Not*I. (see Fig. 5)

EXAMPLE VII : Preparation of PHB-producing recombinant *Alcaligenes eutrophus* LAR5

The recombinant expression vector pKTC32 of Example VI was introduced into the strains of *A. eutrophus* DSM541 which is lacking in PHB gene. When culturing the transformant, PHB particles in the cell were observed.

EXAMPLE VIII : Identification of primer region of PHB gene derived from *A. latus*

For the purpose of identifying the PHB primer region, the total DNA of *Alcaligenes latus* was separated. The site wherefrom RNA transcription starts was determined by primer extension method and then the promoter region consisting of 210 bp DNA upstream was obtained. The gene sequence of promoter region of PHB was analyzed by PC/Gene software program .

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO: Lee, Sang Yup
Expo Apt. 212-702, Chunmin-dong, Yusong-ku, Taejon 305-390,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Escherichia coli* XL1-Blue/pJC1	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCTC 0398BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: [x] a scientific description [ ] a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November 5 1997.	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korea Research Institute of Bioscience and Biotechnology Korean Collection for Type Cultures Address: KCTC, KRIBB #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Bae K.S. Kyung Sook Bae, Curator Date: November 12 1997

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO: LEE, Yong-Hyun
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Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: ***Alcaligenes eutrophus LAR5***	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY ***KCTC 0568BP***

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on ***January 18 1999.***

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **Korean Collection for Type Cultures** Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): PARK Yong-Ha, Director Date: ***January 25 1999***

WHAT IS CLAIMED :

1. A polyhydroxyalkanoate biosynthesis-related DNA fragment, comprising a gene for polyhydroxyalkanoate synthase, a gene for β-ketothiolase and a gene for acetoacetyl-CoA reductase, which are all derived from *Alcaligenes latus*.

2. A polyhydroxyalkanoate biosynthesis-related DNA fragment as set forth in claim 1, wherein said fragment contain the gene for polyhydroxyalkanoate synthase, the gene for β-ketothiolase and the gene for acetoacetyl-CoA reductase in due order and has a base sequence of Sequence 1.

3. A polyhydroxyalkanoate biosynthesis-related DNA fragment as set forth in claim 1 or 2, wherein the gene for polyhydroxyalkanoate synthase has a base sequence of Sequence 2.

4. A polyhydroxyalkanoate biosynthesis-related DNA fragment as set forth in claim 1 or 2, wherein the gene for β-ketothiolase has a base sequence of Sequence 3.

5. A polyhydroxyalkanoate biosynthesis-related DNA fragment as set forth in claim 1 or 2, wherein the gene for acetoacetyl-CoA reductase has a base sequence of Sequence 4.

6. A polyhydroxyalkanoate synthase, having an amino acid sequence of Sequence 5, derived from *Alcaligenes latus*.

7. A β-ketothiolase, having an amino acid sequence of Sequence 6, derived from *Alcaligenes latus*.

8. An acetoacetyl-CoA reductase, having an amino acid sequence of Sequence 7, derived from *Alcaligenes latus*.

9. A recombinant expression vector pJC1, containing the polyhydroxyalkanoate biosynthesis-related gene of claim 1.

10. A recombinant expression vector pAL32, containing the gene for polyhydroxyalkanoate synthase of claim 3.

11. . A recombinant expression vector pKTC32, containing the gene for polyhydroxyalkanoate synthase of claim 3.

12. An *E. coli* transformant XL1-Blue/pJC1 with a deposition No. of KCTC 0398 BP, which is transformed with the recombinant expression vector of claim 9.

13. An *Alcaligenes eutrophus* transformant LAR5 (DSM541/pKTC32) with a deposition No. KCTC 0568 BP, which is transformed with the recombinant expression vector of claim 11.

14. A method for preparing polyhydroxyalkanoate biosynthesis-related enzymes, by culturing the *E. coli* transformant of claim 12.

15. A method for preparing polyhydroxybutyrate synthase, by culturing *A. eutrophus* transformant of claim 13.

16. A method for producing polyhydroxyalkanoate and its copolymers, by culturing the transformant of claim 12.

17. A method for producing polyhydroxyalkanoate and its copolymers, by culturing the transformant of 13.

FIG. 1

FIG. 2

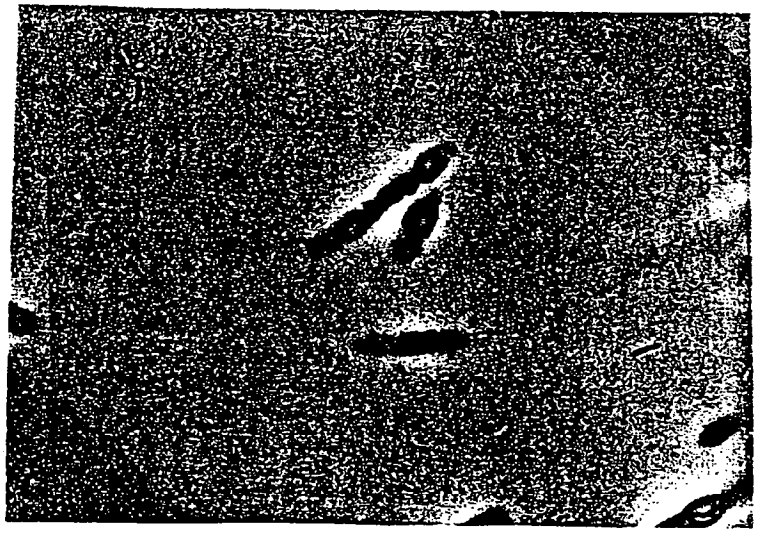

FIG. 3a

```
        10         20         30         40         50         60
GGATCCTGCT GCGCTCGGAC AAAAGCATGG GCCGAGTTTA GCGCGCGCCC TCGGACGCCC
        70         80         90        100        110        120
CCGGCAGCGT GCAGGGTTCA CGCCATGTTC AAAAGCGCTG TGAGGCAGGT ATGCTGCACT
       130        140        150        160        170        180
GCGTCAATCC CGCAGTTCCG CAGTCATCCC AGAAATGCAG CTGTACAACT ACTTTCGCTC
       190        200        210        220        230        240
CTCGGCGTCC TACCGCGTCC GCATCGCACT GGCCCTGAAG GGTCTGGCCT ACGAATACAA
       250        260        270        280        290        300
GCCGNTGCAC CTGCAGAAGA AGGAGCAGTT CGCGGANTCG TATGCGGCCG TGTCGGCCTC
       310        320        330        340        350        360
GCGCCTGGTG CCGCTGCTGC GCGACGGCGA CGCGTCGCTG ACGCAGTCGA TGGCCATCAT
       370        380        390        400        410        420
CGAGTACCTG GACGAGACCC ATCCGCAGCC GCCGCTGCTG CCCTCGGACC CGCTGGGCCG
       430        440        450        460        470        480
CGCCCGCGTG CGTGCGCTGG CGCAGGACAT CGCCTGCGAG ATCCACCCGC TCAACAACCT
       490        500        510        520        530        540
GCGCGTGCTG CGCTACCTGG CGCACGACCT CAAGGTCGGC GAGGACGACA AGAACCGCTG
       550        560        570        580        590        600
GTACCGCCAC TGGGTCGAGA CCGGCCTGGA GGTGGTGGAG CGCCAGCTGG CGGATCACCC
       610        620        630        640        650        660
GTCCACCGGC CGCTTCTGCC ATGGCGACAC GCCCGGCCTG GCCGATTGCG TGCTGGTGCC
       670        680        690        700        710        720
GCAGATCTTC AACGCCCAGC GTTTCAACTG CCGGCTGGAG CACGTGCCCA CCGTGATGCG
       730        740        750        760        770        780
CGTGTACGAG GCCTGCATGC AGCTCGACGC CTTCGACAAG ACGCAGCCCT CCGCCTGTCC
       790        800        810        820        830        840
CGATGCCGAG TAAGGCTCTG CAGGGCGTGC TGAGGCCCGA GTGGCCGGCA CCGGCCGGCG
       850        860        870        880        890        900
TGGGCGCATT CATGAGCACG CGCGAGGGCG GCGTCAGCGC CGCGCCCTGG GACGGCGCCA
       910        920        930        940        950        960
ACCTGGGCGA CGCCGTGGGC GACAGCCCGC AGGCTGTGGA CACCAACCGC GCCCGATTCG
       970        980        990       1000       1010       1020
CCGCCGCCGC CGAGGGCGGC ACGCCGGTGT GGCTGCGCCA GGTCCACGGC ACGCGGGTGC
      1030       1040       1050       1060       1070       1080
TGCGATTGCG CGCCGGCGAG GCCTTGCCGG CGCAGCCGCC CGAGGCCGAT GCCGTGGTCA
      1090       1100       1110       1120       1130       1140
CCGCCGACCC CGGCCTGGTG TGCGTGGTGC AGGTGGCGGA CTGCCTGCCC GTGTTCTTCG
      1150       1160       1170       1180       1190       1200
CAGCGTCCAA CGGCCGTGCC GTCGGCGCTG CGCATGCGGG CTGGCGCGGC CTGGCCGGTG
      1210       1220       1230       1240       1250       1260
GCGTGCTCGA AAACACGCTG GCCGAGGTGT GCGCGCTGGC GCGCTGCGAG CCCTCCGATG
      1270       1280       1290       1300       1310       1320
TGCTGGCCTG GATGGGGCCC TGCATCGGGC CGGAGAGTTT CGAGGTGGGG CGCGACGTGC
      1330       1340       1350       1360       1370       1380
TGGAGGGTTT CGGCGTGGAT CCGGACGGTC CGGCCGACCC GGCCTTCGCC TGGCGTCCGC
      1390       1400       1410       1420       1430       1440
GTGCCGACGG CAGCGCGCGC TGGCTGGCGG ACCTGCCGGG GCTGGCGCGG CGCCGGCTCG
      1450       1460       1470       1480       1490       1500
AATTGGCAGG TCTGCGTCAG ATCAGTGGCG GACAGTGGTG CACGGTGCAG GATCGTTCAC
      1510       1520       1530       1540       1550       1560
GGTTCTTCTC GTTCCGGCGG GACCGGGTCA CGGGGCGGCA GGCTGCCGCC GTCTGGCTGC
      1570       1580       1590       1600       1610       1620
GCGGATGAAG CGGTGTCCTC GGCGCGCTTG CGCGCCCGTC GCCGCGCCGG CGTCCCCAGG
      1630       1640       1650       1660       1670       1680
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FIG. 3b

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AAGTACAGGA CGATGGACAA GGGCAGTACG CCATACAGCA GCAGCGTGAA CACCGCGCCG
        1690        1700        1710        1720        1730        1740
AGCAAGGTGC CGTTGGGCGC CATGGCTTCG GCCACGGCCA TCATCAGCAC CACGTACAGC
        1750        1760        1770        1780        1790        1800
CATGCCAGAG CAACCAAGTA CATAGCAAAA ACCCGCAATT ACGCAGAATG ACGTATTTCG
        1810        1820        1830        1840        1850        1860
TACAATGAAA ACTGTTGTCA TGATGCGGTA AGACACGAAG CCTACAACGC GATCCAGCAA
        1870        1880        1890        1900        1910        1920
CGGTTTTCGT GAAAAAGTCC TCAGGAGACG AGCGTGACAC TGCATCCCAT TCCCGCACTG
        1930        1940        1950        1960        1970        1980
CAACAGCTTG GCGACAACGC CACGGCGCTG AGTGCCGCCA TCTCGGAAGC GCTGCGCGCG

        1989        1998        2007        2016        2025        2034
ATG TCG GGC CTG AAC CTG CCG ATG CAG GCC ATG ACC AAG CTG CAG GGC GAG TAC
 M  S  G  L  N  L  P  M  Q  A  M  T  K  L  Q  G  E  Y
phaC_Al →

        2043        2052        2061        2070        2079        2088
CTC AAC GAG GCG ACG GCG CTG TGG AAC CAG ACG CTG GGC CGC CTG CAG CCC GAC
 L  N  E  A  T  A  L  W  N  Q  T  L  G  R  L  Q  P  D

        2097        2106        2115        2124        2133        2142
GGC AGC GCC CAA CCG GCC AAG CTG GGC GAC CGG CGC TTC TCG GCC GAG GAC TGG
 G  S  A  Q  P  A  K  L  G  D  R  R  F  S  A  E  D  W

        2151        2160        2169        2178        2187        2196
GCC AAG AAC CCC GCC GCG GCC TAC CTG GCG CAG GTC TAC CTG CTC AAT GCC CGC
 A  K  N  P  A  A  A  Y  L  A  Q  V  Y  L  L  N  A  R

        2205        2214        2223        2232        2241        2250
ACG CTG ATG CAG ATG GCC GAG TCC ATC GAG GGC GAC GCC AAG GCC AAG GCG CGC
 T  L  M  Q  M  A  E  S  I  E  G  D  A  K  A  K  A  R

        2259        2268        2277        2286        2295        2304
GTG CGC TTC GCC GTG CAG CAG TGG ATC GAC GCC GCG GCG CCG AGC AAC TTC CTG
 V  R  F  A  V  Q  Q  W  I  D  A  A  A  P  S  N  F  L

        2313        2322        2331        2340        2349        2358
GCG CTC AAT CCC GAG GCG CAG CGC AAG GCG CTG GAG ACC AAG GGG GAG AGC ATC
 A  L  N  P  E  A  Q  R  K  A  L  E  T  K  G  E  S  I

        2367        2376        2385        2394        2403        2412
AGC CAG GGC CTG CAG CAG CTG TGG CAT GAC ATC CAG CAG GGC CAC GTG TCG CAG
 S  Q  G  L  Q  Q  L  W  H  D  I  Q  Q  G  H  V  S  Q

        2421        2430        2439        2448        2457        2466
ACG GAC GAG AGC GTG TTC GAG GTG GGC AAG AAC GTC GCC ACC ACC GAG GGC GCG
 T  D  E  S  V  F  E  V  G  K  N  V  A  T  T  E  G  A

        2475        2484        2493        2502        2511        2520
GTC GTG TAC GAG AAC GAC CTG TTC CAG CTC ATC GAG TAC AAG CCG CTG ACG CCC
 V  V  Y  E  N  D  L  F  Q  L  I  E  Y  K  P  L  T  P

        2529        2538        2547        2556        2565        2574
AAG GTG CAC GAG AAG CCG ATG CTG TTC GTG CCG CCG TGC ATC AAC AAG TAC TAC
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FIG. 3c

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K  V  H  E  K  P  M  L  F  V  P  P  C  I  N  K  Y  Y

        2583        2592        2601        2610        2619        2628
ATC CTG GAC CTG CAG CCG GAC AAC AGC CTC ATC CGC TAC ACC GTC GCC CAG GGC
I  L  D  L  Q  P  D  N  S  L  I  R  Y  T  V  A  Q  G

        2637        2646        2655        2664        2673        2682
CAC CGG GTG TTC GTG GTG AGC TGG CGC AAC CCC GAC GCC TCC GTC GCC GGC AAG
H  R  V  F  V  V  S  W  R  N  P  D  A  S  V  A  G  K

        2691        2700        2709        2718        2727        2736
ACC TGG GAC GAC TAC GTG GAG CAG GGC GTG ATC CGC GCC ATC CGC GTG ATG CAG
T  W  D  D  Y  V  E  Q  G  V  I  R  A  I  R  V  M  Q

        2745        2754        2763        2772        2781        2790
CAG ATC ACG GGG CAC GAG AAG GTC AAC GCG CTG GGC TTC TGC GTC GGC GGC ACC
Q  I  T  G  H  E  K  V  N  A  L  G  F  C  V  G  G  T

        2799        2808        2817        2826        2835        2844
ATC CTG AGC ACG GCG CTG GCG GTG CTG GCC GCG CGC GGC GAG CAG CCC GCG GCG
I  L  S  T  A  L  A  V  L  A  A  R  G  E  Q  P  A  A

        2853        2862        2871        2880        2889        2898
AGC CTG ACG CTG CTG ACC ACG CTG CTG GAC TTC AGC AAC ACC GGC GTG CTG GAC
S  L  T  L  L  T  T  L  L  D  F  S  N  T  G  V  L  D

        2907        2916        2925        2934        2943        2952
CTG TTC ATC GAC GAG GCC GGC GTG CGC CTG CGC GAG ATG ACC ATC GGC GAG AAG
L  F  I  D  E  A  G  V  R  L  R  E  M  T  I  G  E  K

        2961        2970        2979        2988        2997        3006
GCG CCC AAC GGC CCG GGC CTG CTC AAC GGC AAG GAG CTG GCC ACC ACC TTC AGC
A  P  N  G  P  G  L  L  N  G  K  E  L  A  T  T  F  S

        3015        3024        3033        3042        3051        3060
TTC CTG CGC CCG AAC GAC CTG GTC TGG AAC TAC GTG GTG GGC AAC TAC CTC AAG
F  L  R  P  N  D  L  V  W  N  Y  V  V  G  N  Y  L  K

        3069        3078        3087        3096        3105        3114
GGC GAG GCG CCG CCG CCC TTC GAC CTG CTG TAC TGG AAC TCC GAC AGC ACC AAC
G  E  A  P  P  P  F  D  L  L  Y  W  N  S  D  S  T  N

        3123        3132        3141        3150        3159        3168
ATG GCC GGG CCC ATG TTC TGC TGG TAC CTG CGC AAC ACC TAC CTG GAG AAC AAG
M  A  G  P  M  F  C  W  Y  L  R  N  T  Y  L  E  N  K

        3177        3186        3195        3204        3213        3222
TTG CGC GTT CCC GGT GCC CTG ACC ATC TGC GGC GAG AAG GTG GAC CTC TCG CGC
L  R  V  P  G  A  L  T  I  C  G  E  K  V  D  L  S  R

        3231        3240        3249        3258        3267        3276
ATC GAG GCG CCG GTG TAC TTC TAC GGT TCG CGC GAG GAC CAC ATC GTG CCC TGG
I  E  A  P  V  Y  F  Y  G  S  R  E  D  H  I  V  P  W

        3285        3294        3303        3312        3321        3330
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FIG. 3d

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GAA TCG GCC TAC GCC GGC ACG CAG ATG CTG AGC GGC CCC AAG CGC TAT GTC CTG
 E   S   A   Y   A   G   T   Q   M   L   S   G   P   K   R   Y   V   L

        3339        3348        3357        3366        3375        3384
GGT GCG TCT GGC CAC ATC GCC GGC GTG ATC AAC CCC CCG CAG AAG AAG AAG CGC
 G   A   S   G   H   I   A   G   V   I   N   P   P   Q   K   K   K   R

        3393        3402        3411        3420        3429        3438
AGC TAC TGG ACC AAC GAG CAG CTC GAC GGC GAC TTC AAC CAG TGG CTG GAA GGC
 S   Y   W   T   N   E   Q   L   D   G   D   F   N   Q   W   L   E   G

        3447        3456        3465        3474        3483        3492
TCC ACC GAG CAT CCT GGC AGC TGG TGG ACC GAC TGG AGC GAC TGG CTC AAG CAG
 S   T   E   H   P   G   S   W   W   T   D   W   S   D   W   L   K   Q

        3501        3510        3519        3528        3537        3546
CAC GCG GGC AAG GAA ATC GCC GCA CCC AAG ACT CCC GGC AAC AAG ACC CAC AAG
 H   A   G   K   E   I   A   A   P   K   T   P   G   N   K   T   H   K

        3555        3564        3573        3582
CCC ATC GAG CCC GCC CCC GGG CGT TAC GTG AAG CAG AAG GCC
 P   I   E   P   A   P   G   R   Y   V   K   Q   K   A

              3600        3610        3620        3630        3640
TG AGCCGCGGCC CCTGAGCCTT CTTTAACCCG ACCTTGACAA ACGAGGAGAT AAGC

        3653        3662        3671        3680        3689        3698
ATG ACC GAC ATC GTC ATC GTC GCC GCA GCC CGC ACC GCC GTG GGC AAG TTC GGC
 M   T   D   I   V   I   V   A   A   A   R   T   A   V   G   K   F   G
phaA_Al →

        3707        3716        3725        3734        3743        3752
GGC ACG CTG GCC AAG ACC CCC GCT CCG GAG CTG GGC GCC GTG GTC ATC AAG GCC
 G   T   L   A   K   T   P   A   P   E   L   G   A   V   V   I   K   A

        3761        3770        3779        3788        3797        3806
CTG CTG GAG AAG ACG GGC GTC AAG CCC GAC CAG ATC GGT GAA GTC ATC ATG GGC
 L   L   E   K   T   G   V   K   P   D   Q   I   G   E   V   I   M   G

        3815        3824        3833        3842        3851        3860
CAG GTG CTG GCC GCC GGC GCG GGC CAG AAC CCC GCG CGC CAG GCG ATG ATG AAG
 Q   V   L   A   A   G   A   G   Q   N   P   A   R   Q   A   M   M   K

        3869        3878        3887        3896        3905        3914
GCG GGC ATC GCC AAG GAA ACG CCG GCG CTG ACC ATC AAC GCC GTG TGC GGG TCC
 A   G   I   A   K   E   T   P   A   L   T   I   N   A   V   C   G   S

        3923        3932        3941        3950        3959        3968
GGC CTC AAG GCC GTG ATG CTG GCC GCC CAG GCC ATC GCC TGG GGC GAC AGC GAC
 G   L   K   A   V   M   L   A   A   Q   A   I   A   W   G   D   S   D

        3977        3986        3995        4004        4013        4022
ATC GTC ATC GCC GGC GGC CAG GAG AAC ATG AGC GCC AGC CCG CAC GTG CTG ATG
 I   V   I   A   G   G   Q   E   N   M   S   A   S   P   H   V   L   M
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FIG. 3e

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        4031        4040        4049        4058        4067        4076
GGC AGC CGC GAC GGC CAG CGC ATG GGC GAC TGG AAG ATG GTC GAC ACC ATG ATC
 G   S   R   D   G   Q   R   M   G   D   W   K   M   V   D   T   M   I

        4085        4094        4103        4112        4121        4130
AAC GAC GGC CTG TGG GAC GTG TAC AAC AAG TAC CAC ATG GGC ATC ACG GCC GAG
 N   D   G   L   W   D   V   Y   N   K   Y   H   M   G   I   T   A   E

        4139        4148        4157        4166        4175        4184
AAC GTC GCC AAG GAA CAC GAC ATC AGC CGC GAC CAG CAG GAC GCC CTG GCC CTG
 N   V   A   K   E   H   D   I   S   R   D   Q   Q   D   A   L   A   L

        4193        4202        4211        4220        4229        4238
GCC AGC CAG CAG AAG GCC ACC GCC GCG CAG GAA GCC GGC CGC TTC AAG GAC GAG
 A   S   Q   Q   K   A   T   A   A   Q   E   A   G   R   F   K   D   E

        4247        4256        4265        4274        4283        4292
ATC GTT CCG GTC TCG ATC CCG CAG CGC AAG GGC GAC CCG GTG CTG TTC GAC ACC
 I   V   P   V   S   I   P   Q   R   K   G   D   P   V   L   F   D   T

        4301        4310        4319        4328        4337        4346
GAC GAG TTC ATC AAC AAG AAG ACC ACC GCC GAA GCG CTG GCG GGC CTG CGC CCG
 D   E   F   I   N   K   K   T   T   A   E   A   L   A   G   L   R   P

        4355        4364        4373        4382        4391        4400
GCC TTC GAC AAG GCC GGC AGC GTG ACC GCG GGC AAC GCC TCG GGC ATC AAC GAC
 A   F   D   K   A   G   S   V   T   A   G   N   A   S   G   I   N   D

        4409        4418        4427        4436        4445        4454
GGC GCC GCT GCG GTG ATG GTG ATG TCC GCC GCC AAG GCG AAG GAG CTG GGC CTG
 G   A   A   A   V   M   V   M   S   A   A   K   A   K   E   L   G   L

        4463        4472        4481        4490        4499        4508
ACG CCC ATG GCG CGC ATC AAG AGC TTC GGC ACC AGC GGC CTG GAT CCG GCC AAG
 T   P   M   A   R   I   K   S   F   G   T   S   G   L   D   P   A   K

        4517        4526        4535        4544        4553        4562
GTC AAC GTC AAC GGC GGT GCC ATC GCC ATC GGC CAC CCC ATC GGC GCC TCC GGC
 V   N   V   N   G   G   A   I   A   I   G   H   P   I   G   A   S   G

        4571        4580        4589        4598        4607        4616
TGC CGC GTG CTG GTG ACG CTG CTG CAC GAG ATG CAG CGC CGG GAC GCC AAG AAG
 C   R   V   L   V   T   L   L   H   E   M   Q   R   R   D   A   K   K

        4625        4634        4643        4652        4661        4670
GGC CTG GCC GCG CTG TGC ATC GGC GGC GGC ATG GGC GTG TCG CTG ACC GTC GAG
 G   L   A   A   L   C   I   G   G   G   M   G   V   S   L   T   V   E

CGC
 R

    4680        4690        4700        4710        4720        4730
TGATCAG AAGAACCGGG CGGCCCCGCG CCGCCCGCCC GGCGTTCCAC GCGGGTGCGC
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FIG. 3f

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        4740        4750        4760        4770        4780        4790
CGGGATACCA GACGAACCAA ACCACCAAGG GCTTCGAGAC GGCCCGAAGA AGGAGAGACA

G

        4800        4809        4818        4827        4836        4845
ATG GCA CAG AAA CTG GCT TAC GTG ACC GGC GGC ATG GGC GGC ATC GGC ACC TCG
 M   A   Q   K   L   A   Y   V   T   G   G   M   G   G   I   G   T   S
pliaB_AI →

        4854        4863        4872        4881        4890        4899
ATG TGC CAG CGC CTG CAC AAG GAC GGC TTC AAG GTG ATC GCC GGC TGC GGT CCG
 M   C   Q   R   L   H   K   D   G   F   K   V   I   A   G   C   G   P

        4908        4917        4926        4935        4944        4953
AGC CGC GAC CAC CAG AAG TGG ATC GAT GAA CAG GCC GCG CTG GGC TAT ACC TTC
 S   R   D   H   Q   K   W   I   D   E   Q   A   A   L   G   Y   T   F

        4962        4971        4980        4989        4998        5007
TAC GCC TCC GTG GGC AAC GTG GCC GAC TGG GAC TCC ACC GTG GCC GCC TTC GAG
 Y   A   S   V   G   N   V   A   D   W   D   S   T   V   A   A   F   E

        5016        5025        5034        5043        5052        5061
AAG GTC AAG GCC GAG CAC GGC ACC GTG GAC GTG CTG GTG AAC AAC GCC GGC ATC
 K   V   K   A   E   H   G   T   V   D   V   L   V   N   N   A   G   I

        5070        5079        5088        5097        5106        5115
ACG CGT GAC GGG CAG TTC CGC AAG ATG AGC AAG GCC GAT TGG CAG GCC GTG ATG
 T   R   D   G   Q   F   R   K   M   S   K   A   D   W   Q   A   V   M

        5124        5133        5142        5151        5160        5169
TCG ACC AAC CTC GAC AGC ATG TTC AAC GTC ACC AAG CAG GTG ATC GAG GGC ATG
 S   T   N   L   D   S   M   F   N   V   T   K   Q   V   I   E   G   M

        5178        5187        5196        5205        5214        5223
CTG GAC AAG GGC TGG GGC CGG ATC ATC AAC ATC TCC TCG GTC AAC GGC GAG AAG
 L   D   K   G   W   G   R   I   I   N   I   S   S   V   N   G   E   K

        5232        5241        5250        5259        5268        5277
GGC CAG TTC GGC CAG ACC AAC TAC TCC GCC GCC AAG GCC GGC ATG CAC GGC TTC
 G   Q   F   G   Q   T   N   Y   S   A   A   K   A   G   M   H   G   F

        5286        5295        5304        5313        5322        5331
TCC ATG GCG CTG GCG CAG GAA GTG GCG GCC AAG GGC GTG ACG GTG AAC ACC GTG
 S   M   A   L   A   Q   E   V   A   A   K   G   V   T   V   N   T   V

        5340        5349        5358        5367        5376        5385
AGC CCG GGC TAC ATC GCC ACG GAC ATG GTC AAG GCC ATC CGC CAG GAC GTG CTG
 S   P   G   Y   I   A   T   D   M   V   K   A   I   R   Q   D   V   L

        5394        5403        5412        5421        5430        5439
GAC AAG ATC ATC GCC ACC ATT CCC ATC CGT CGC CTG GGT ACG CCG GAG GAG ATC
 D   K   I   I   A   T   I   P   I   R   R   L   G   T   P   E   E   I

        5448        5457        5466        5475        5484        5493
```

FIG. 3g

```
GCC TCC ATC TTC CCC TGG CTG GCC GGC GAA GAA TCG GGC TTC ACC ACC GGT GCC
 A   S   I   F   P   W   L   A   G   E   E   S   G   F   T   T   G   A

        5502            5511            5520
GAC TTC AGC TGC AAC GGC GGC CTG CAC ATG GGC
 D   F   S   C   N   G   G   L   H   M   G

 5530            5540            5550            5560            5570            5580
TGAG GCCCGCGGCT CCATGCCCAC CTGCGTGGGC ATGGACGGGC CGAAGGACCG
        5590            5600            5610            5620            5630            5640
AGCTCTGCGA GGGTGCGGCC TGCAAGGCTG AGGCCTGCTG CGCCGCGTGC CCGCGAGGGC
        5650            5660            5670            5680            5690            5700
ACGTGCCGAA GCACCAAAAG GCCGCGCATT GCGCGGCCTT TTCCTTTCTG GATCGGTGCG
        5710            5720            5730            5740            5750            5760
GACGGGTGCC GCGTCAGGCA GGGCAGGCCC CCGGCCTTCA CTCCACCATG CCGGACATGA
        5770            5780            5790            5800            5810            5820
AGTACTTGAT CACCCTTTGG CCGCGAAGCC CAGCATGCCG AAGCCCAGCG CCAGGAACAG
        5830            5840            5850            5860            5870            5880
CACGAAGGTG CCGAACTTGC CGGCCTTCGA CTCGCGCGCG AGCTGAAAGA TGATGAATGC
        5890            5900            5910            5920            5930            5940
CATGTAGAGC ATGAAGGCCG TGACGCCGAC GGTCAGGCCC AGCTGGGCAA TGTTTTCCTC
        5950            5960            5970            5980            5990            6000
GTTGATTTCG AACATCGTTT GTTGTCTCAG GCTGCTGCCA CGCGGCTGAC GTGCTCGCCG
        6010            6020            6030            6040            6050            6060
CGCGGCCGGG CCCCAACTGC CCGCAGCGGT TCTCGATCAG GTTCTCAAGG CATCTCGTGC
        6070            6080            6090            6100            6110            6120
CACTGGGAGG TGTCCACCAG GTCGCGGTAG GCGTGCCAGC TCGAATGCGC CAGCCACGGC
        6130            6140            6150            6160            6170            6180
ACTACCACGA TCAGGCCCAG CAGCAGCGTG GCCATGCCCA GCAGCGTCAG CGCCATGATC
        6190            6200            6210            6220            6230            6240
AGCGCCGCCC ACAGCGCCAG CGGCAGTGGG TGCTGCATCA CCACGCGCCA GCTCGTGAGC
        6250            6260            6270            6280
ACCGCCACCA GCACGCCCAC GTGGCGGTCC AGCAGCATCG GGATCC
```

FIG. 4

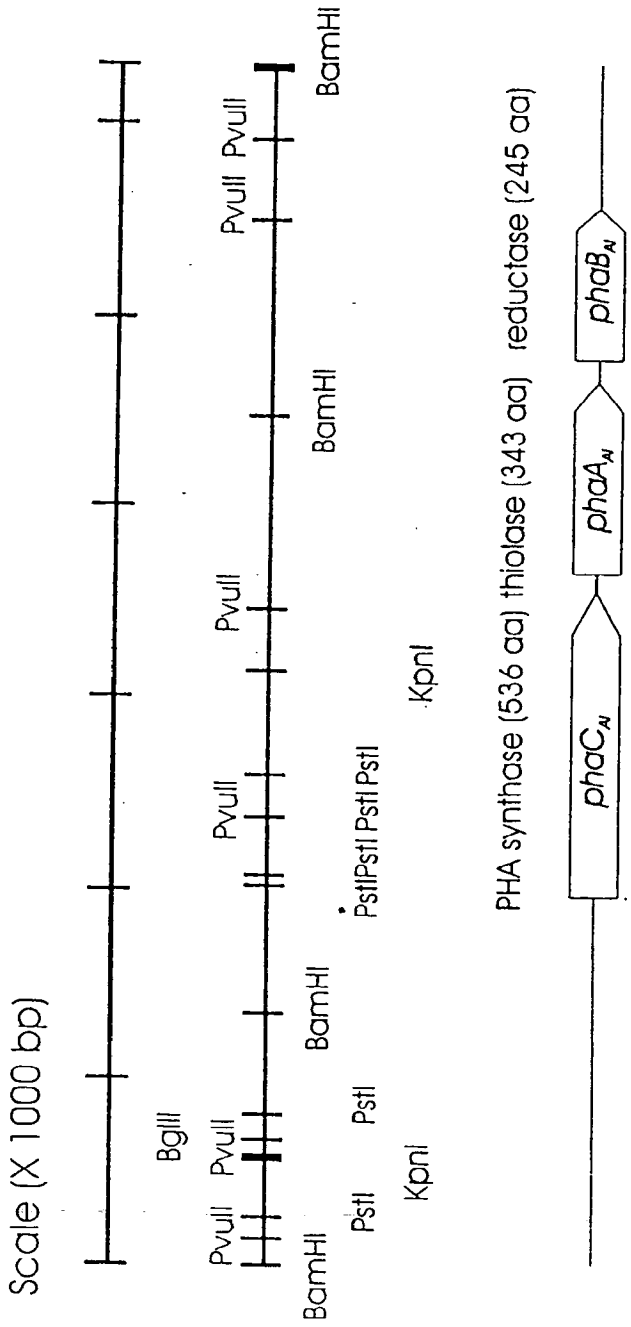


Fig. 5

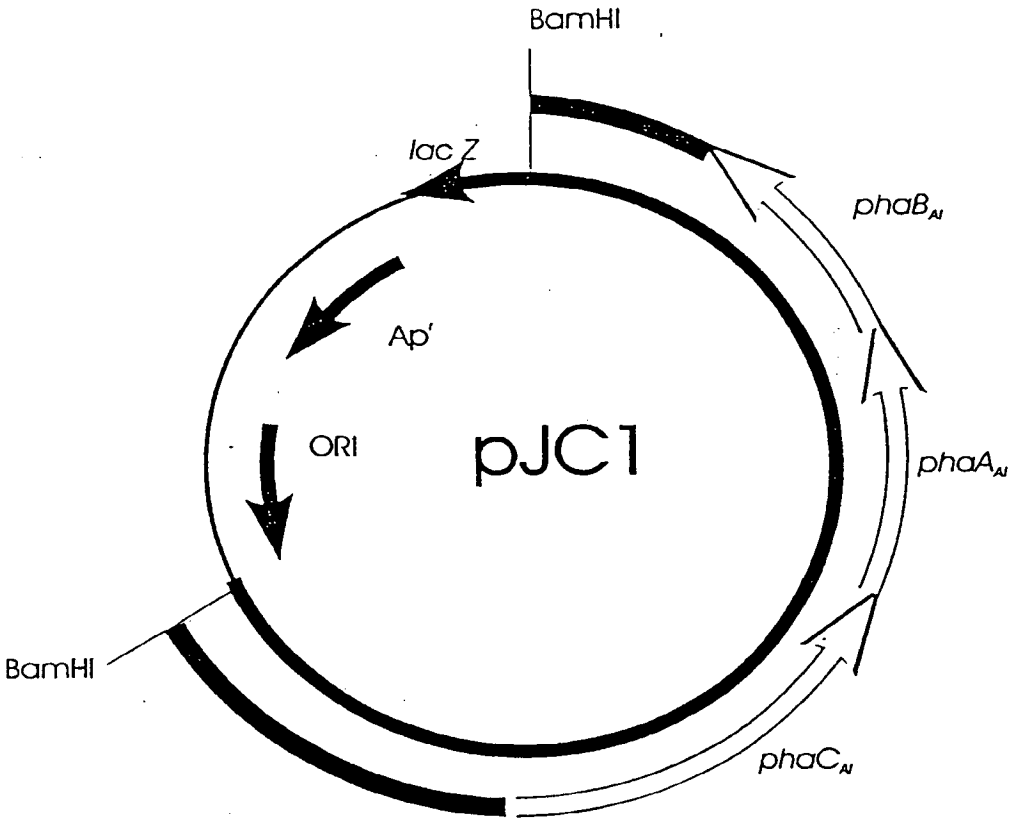
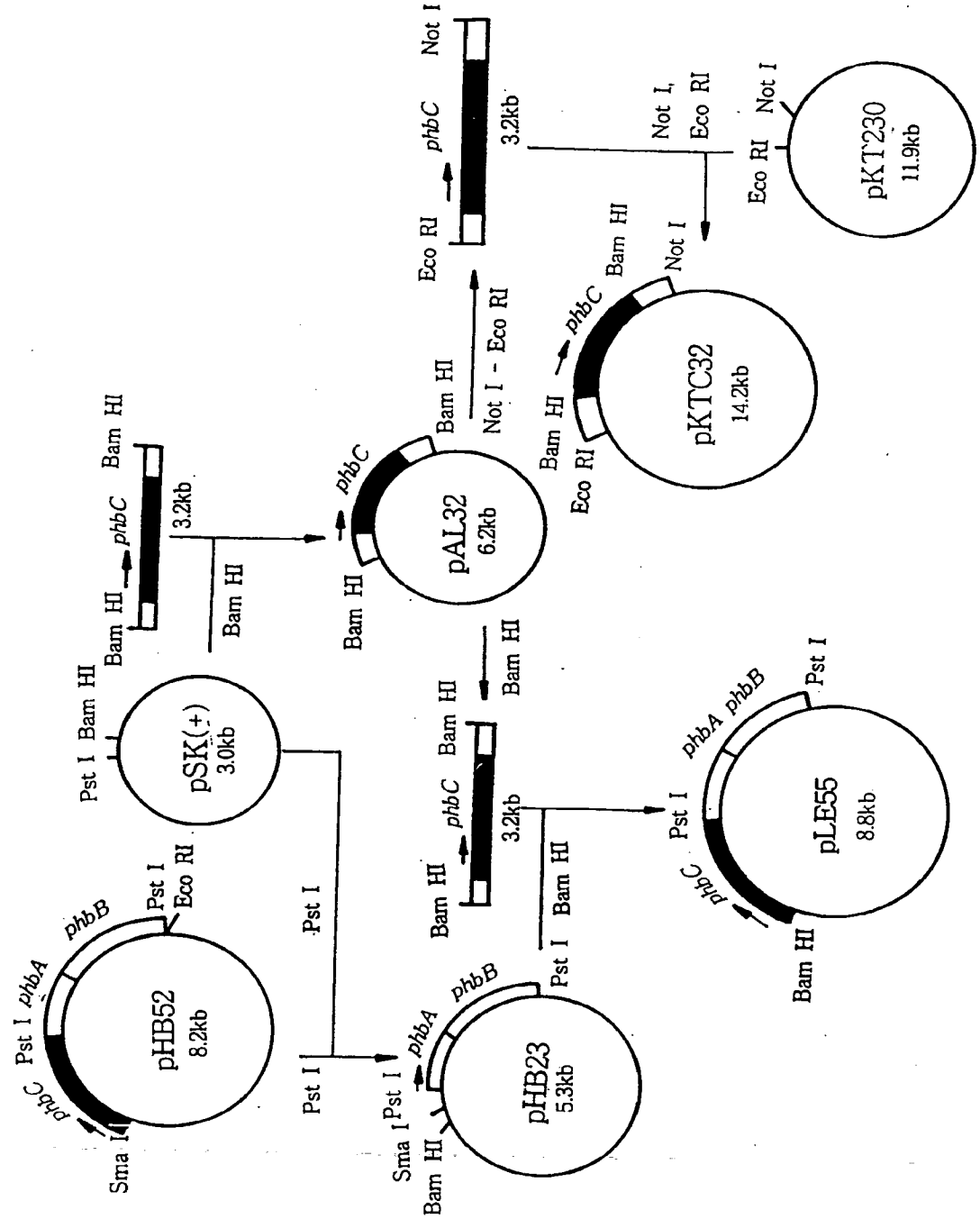


Fig. 6



SEQUENCE LISTING

(1) GENERAL INFORMATION :

(i) APPLICANT : LG CHEMICAL LTD.

LEE, Sang Yup

CHOI, Jong-il

CHOO, Seung-Ho

YOON, Hye-Sung

HAN, Kyuboem

SONG, Ji-Yong

LEE, Yong-Hyun

HUH, Tae-Lin

HONG, Sung-Kook

(ii) TITLE OF INVENTION : POLYHYDROXYALKANOATE BIOSYNTHESIS-RELATED GENES DERIVED FROM *Alcaligenes latus*

(iii) NUMBER OF SEQUENCES : 8

(2) INFORMATION FOR SEQ ID NO. : 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 6436 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : oligonucleotide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 1:

GGATCCTGCT GCGCTCGGAC AAAAGCATGG GCCGAGTTTA GCGCGCGCCC TCGGACGCCC 60
CCGGCAGCGT GCAGGGTTCA CGCCATGTTC AAAAGCGCTG TGAGGCAGGT ATGCTGCACT 120
GCGTCAATCC CGCAGTTCCG CAGTCATCCC AGAAATGCAG CTGTACAACT ACTTTCGCTC 180
CTCGGCGTCC TACCGCGTCC GCATCGCACT GGCCCTGAAG GGTCTGGCCT ACGAATACAA 240
GCCGGTGCAC CTGCAGAAGA AGGAGCAGTT CGCGGAGTCG TATGCGGCCG TGTCGGCCTC 300
GCGCCTGGTG CCGCTGCTGC GCGACGGCGA CGCGTCGCTG ACGCAGTCGA TGGCCATCAT 360
CGAGTACCTG GACGAGACCC ATCCGCAGCC GCCGCTGCTG CCCTCGGACC CGCTGGGCCG 420
CGCCCGCGTG CGTGCGCTGG CGCAGGACAT CGCCTGCGAG ATCCACCCGC TCAACAACCT 480
GCGCGTGCTG CGCTACCTGG CGCACGACCT CAAGGTCGGC GAGGACGACA AGAACCGCTG 540
GTACCGCCAC TGGGTCGAGA CCGGCCTGGA GGTGGTGGAG CGCCAGCTGG CGGATCACCC 600
GTCCACCGGC CGCTTCTGCC ATGGCGACAC GCCCGGCCTG GCCGATTGCG TGCTGGTGCC 660
GCAGATCTTC AACGCCCAGC GTTTCAACTG CCGGCTGGAG CACGTGCCCA CCGTGATGCG 720
CGTGTACGAG GCCTGCATGC AGCTCGACGC CTTCGACAAG ACGCAGCCCT CCGCCTGTCC 780
CGATGCCGAG TAAGGCTCTG CAGGGCGTGC TGAGGCCCGA GTGGCCGGCA CCGGCCGGCG 840
TGGGCGCATT CATGAGCACG CGCGAGGGCG GCGTCAGCGC CGCGCCCTGG GACGGCGCCA 900
ACCTGGGCGA CGCCGTGGGC GACAGCCCGC AGGCTGTGGA CACCAACCGC GCCCGATTCG 960
CCGCCGCCGC CGAGGGCGGC ACGCCGGTGT GGCTGCGCCA GGTCCACGGC ACGCGGGTGC 1020
TGCGATTGCG CGCCGGCGAG GCCTTGCCGG CGCAGCCGCC CGAGGCCGAT GCCGTGGTCA 1080
CCGCCGACCC CGGCCTGGTG TGCGTGGTGC AGGTGGCGGA CTGCCTGCCC GTGTTCTTCG 1140
CAGCGTCCAA CGGCCGTGCC GTCGGCGCTG CGCATGCGGG CTGGCGCGGC CTGGCCGGTG 1200
GCGTGCTCGA AAACACGCTG GCCGAGGTGT GCGCGCTGGC GCGCTGCGAG CCCTCCGATG 1260
TGCTGGCCTG GATGGGGCCC TGCATCGGGC CGGAGAGTTT CGAGGTGGGG CGCGACGTGC 1320
TGGAGGGTTT CGGCGTGGAT CCGGACGGTC CGGCCGACCC GGCCTTCGCC TGGCGTCCGC 1380
GTGCCGACGG CAGCGCGCGC TGGCTGGCGG ACCTGCCGGG GCTGGCGCGG CGCCGGCTCG 1440
AATTGGCAGG TCTGCGTCAG ATCAGTGGCG GACAGTGGTG CACGGTGCAG GATCGTTCAC 1500
GGTTCTTCTC GTTCCGGCGG GACCGGGTCA CGGGGCGGCA GGCTGCCGCC GTCTGGCTGC 1560
GCGGATGAAG CGGTGTCCTC GGCGCGCTTG CGCGCCCGTC GCCGCGCCGG CGTCCCCAGG 1620
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AGCAAGGTGC CGTTGGGCGC CATGGCTTCG GCCACGGCCA TCATCAGCAC CACGTACAGC 1740

CATGCCAGAG CAACCAAGTA CATAGCAAAA ACCCGCAATT ACGCAGAATG ACGTATTTCG 1800
TACAATGAAA ACTGTTGTCA TGATGCGGTA AGACACGAAG CCTACAACGC GATCCAGCAA 1860
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ATGTCGGGCC TGAACCTGCC GATGCAGGCC ATGACCAAGC TGCAGGGCGA GTACCTCAAC 2040
GAGGCGACGG CGCTGTGGAA CCAGACGCTG GGCCGCCTGC AGCCCGACGG CAGCGCCCAA 2100
CCGGCCAAGC TGGGCGACCG GCGCTTCTCG GCCGAGGACT GGGCCAAGAA CCCCGCCGCG 2160
GCCTACCTGG CGCAGGTCTA CCTGCTCAAT GCCCGCACGC TGATGCAGAT GGCCGAGTCC 2220
ATCGAGGGCG ACGCCAAGGC CAAGGCGCGC GTGCGCTTCG CCGTGCAGCA GTGGATCGAC 2280
GCCGCGGCGC CGAGCAACTT CCTGGCGCTC AATCCCGAGG CGCAGCGCAA GGCGCTGGAG 2340
ACCAAGGGGG AGAGCATCAG CCAGGGCCTG CAGCAGCTGT GGCATGACAT CCAGCAGGGC 2400
CACGTGTCGC AGACGGACGA GAGCGTGTTC GAGGTGGGCA AGAACGTCGC CACCACCGAG 2460
GGCGCGGTCG TGTACGAGAA CGACCTGTTC CAGCTCATCG AGTACAAGCC GCTGACGCCC 2520
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CAGTTCCGCA AGATGAGCAA GGCCGATTGG CAGGCCGTGA TGTCGACCAA CCTCGACAGC 5280
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CGTGGGCATG GACGGGCCGA AGGACCCGAG CTCTGCGAGG GTGCGGCCTG CAAGGCTGAG 5760
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GCCATGCCCA GCAGCGTCAG CGCCATGATC AGCGCCGCCC ACAGCGCCAG CGGCAGTGGG 6360
TGCTGCATCA CCACGCGCCA GCTCGTGAGC ACCGCCACCA GCACGCCCAC GTGGCGGTCC 6420
AGCAGCATCG GGATCC 6436

(2) INFORMATION FOR SEQ ID NO. : 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1161 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : oligonucleotide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 2:

ATGTCGGGCC TGAACCTGCC GATGCAGGCC ATGACCAAGC TGCAGGGCGA GTACCTCAAC 60
GAGGCGACGG CGCTGTGGAA CCAGACGCTG GGCCGCCTGC AGCCCGACGG CAGCGCCCAA 120
CCGGCCAAGC TGGGCGACCG GCGCTTCTCG GCCGAGGACT GGGCCAAGAA CCCCGCCGCG 180
GCCTACCTGG CGCAGGTCTA CCTGCTCAAT GCCCGCACGC TGATGCAGAT GGCCGAGTCC 240
ATCGAGGGCG ACGCCAAGGC CAAGGCGCGC GTGCGCTTCG CCGTGCAGCA GTGGATCGAC 300
GCCGCGGCGC CGAGCAACTT CCTGGCGCTC AATCCCGAGG CGCAGCGCAA GGCGCTGGAG 360
ACCAAGGGGG AGAGCATCAG CCAGGGCCTG CAGCAGCTGT GGCATGACAT CCAGCAGGGC 420
CACGTGTCGC AGACGGACGA GAGCGTGTTC GAGGTGGGCA AGAACGTCGC CACCACCGAG 480
GGCGCGGTCG TGTACGAGAA CGACCTGTTC CAGCTCATCG AGTACAAGCC GCTGACGCCC 540
AAGGTGCACG AGAAGCCGAT GCTGTTCGTG CCGCCGTGCA TCAACAAGTA CTACATCCTG 600
GACCTGCAGC CGGACAACAG CCTCATCCGC TACACCGTCG CCCAGGGCCA CCGGGTGTTC 660
GTGGTGAGCT GGCGCAACCC CGACGCCTCC GTCGCCGGCA AGACCTGGGA CGACTACGTG 720
GAGCAGGGCG TGATCCGCGC CATCCGCGTG ATGCAGCAGA TCACGGGGCA CGAGAAGGTC 780
AACGCGCTGG GCTTCTGCGT CGGCGGCACC ATCCTGAGCA CGGCGCTGGC GGTGCTGGCC 840
GCGCGCGGCG AGCAGCCCGC GGCGAGCCTG ACGCTGCTGA CCACGCTGCT GGACTTCAGC 900
AACACCGGCG TGCTGGACCT GTTCATCGAC GAGGCCGGCG TGCGCCTGCG CGAGATGACC 960
ATCGGCGAGA AGGCGCCCAA CGGCCCGGGC CTGCTCAACG GCAAGGAGCT GGCCACCACC 1020
TTCAGCTTCC TGCGCCCGAA CGACCTGGTC TGGAACTACG TGGTGGGCAA CTACCTCAAG 1080
GGCGAGGCGC CGCCGCCCTT CGACCTGCTG TACTGGAACT CCGACAGCAC CAACATGGCC 1140
GGGCCCATGT TCTGCTGGTA CCTGCGCAAC ACCTACCTGG AGAACAAGTT GCGCGTTCCC 1200
GGTGCCCTGA CCATCTGCGG CGAGAAGGTG GACCTCTCGC GCATCGAGGC GCCGGTGTAC 1260
TTCTACGGTT CGCGCGAGGA CCACATCGTG CCCTGGGAAT CGGCCTACGC CGGCACGCAG 1320
ATGCTGAGCG GCCCCAAGCG CTATGTCCTG GGTGCGTCTG GCCACATCGC CGGCGTGATC 1380
AACCCCCCGC AGAAGAAGAA GCGCAGCTAC TGGACCAACG AGCAGCTCGA CGGCGACTTC 1440
AACCAGTGGC TGGAAGGCTC CACCGAGCAT CCTGGCAGCT GGTGGACCGA CTGGAGCGAC 1500
TGGCTCAAGC AGCACGCGGG CAAGGAAATC GCCGCACCCA AGACTCCCGG CAACAAGACC 1560
CACAAGCCCA TCGAGCCCGC CCCCGGGCGT TACGTGAAGC AGAAGGCCTG A 1611

(2) INFORMATION FOR SEQ ID NO. : 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1179 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : oligonucleotide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. :3:

ATGACCGACA TCGTCATCGT CGCCGCAGCC CGCACCGCCG TGGGCAAGTT CGGCGGCACG 60
CTGGCCAAGA CCCCCGCTCC GGAGCTGGGC GCCGTGGTCA TCAAGGCCCT GCTGGAGAAG 120
ACGGGCGTCA AGCCCGACCA GATCGGTGAA GTCATCATGG GCCAGGTGCT GGCCGCCGGC 180
GCGGGCCAGA ACCCCGCGCG CCAGGCGATG ATGAAGGCGG GCATCGCCAA GGAAACGCCG 240
GCGCTGACCA TCAACGCCGT GTGCGGCTCC GGCCTCAAGG CCGTGATGCT GGCCGCCCAG 300
GCCATCGCCT GGGGCGACAG CGACATCGTC ATCGCCGGCG GCCAGGAGAA CATGAGCGCC 360
AGCCCGCACG TGCTGATGGG CAGCCGCGAC GGCCAGCGCA TGGGCGACTG GAAGATGGTC 420
GACACCATGA TCAACGACGG CCTGTGGGAC GTGTACAACA AGTACCACAT GGGCATCACG 480
GCCGAGAACG TCGCCAAGGA ACACGACATC AGCCGCGACC AGCAGGACGC CCTGGCCCTG 540
GCCAGCCAGC AGAAGGCCAC CGCCGCGCAG GAAGCCGGCC GCTTCAAGGA CGAGATCGTT 600
CCGGTCTCGA TCCCGCAGCG CAAGGGCGAC CCGGTGCTGT TCGACACCGA CGAGTTCATC 660
AACAAGAAGA CCACCGCCGA AGCGCTGGCG GGCCTGCGCC CGGCCTTCGA CAAGGCCGGC 720
AGCGTGACCG CGGGCAACGC CTCGGGCATC AACGACGGCG CCGCTGCGGT GATGGTGATG 780
TCCGCCGCCA AGGCGAAGGA GCTGGGCCTG ACGCCCATGG CGCGCATCAA GAGCTTCGGC 840
ACCAGCGGCC TGGATCCGGC CACCATGGGC ATGGGCCCGG TGCCGGCCTC GCGCAAGGCG 900
CTGGAGCGCG CCGGCTGGCA GGTCGGTGAC GTGGACCTGT TCGAGCTCAA CGAAGCCTTC 960
GCCGCCCAGG CCTGCGCGGT GAACAAGGAG CTGGGCGTGG ATCCGGCCAA GGTCAACGTC 1020
AACGGCGGTG CCATCGCCAT CGGCCACCCC ATCGGCGCCT CCGGCTGCCG CGTGCTGGTG 1080
ACGCTGCTGC ACGAGATGCA GCGCCGGGAC GCCAAGAAGG GCCTGGCCGC GCTGTGCATC 1140
GGCGGCGGCA TGGGCGTGTC GCTGACCGTC GAGCGCTGA 1179

(2) INFORMATION FOR SEQ ID NO. : 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 738 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : oligonucleotide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 4:

ATGGCACAGA AACTGGCTTA CGTGACCGGC GGCATGGGCG GCATCGGCAC CTCGATGTGC 60
CAGCGCCTGC ACAAGGACGG CTTCAAGGTG ATCGCCGGCT GCGGTCCGAG CCGCGACCAC 120
CAGAAGTGGA TCGATGAACA GGCCGCGCTG GGCTATACCT TCTACGCCTC CGTGGGCAAC 180
GTGGCCGACT GGGACTCCAC CGTGGCCGCC TTCGAGAAGG TCAAGGCCGA GCACGGCACC 240
GTGGACGTGC TGGTGAACAA CGCCGGCATC ACGCGTGACG GGCAGTTCCG CAAGATGAGC 300
AAGGCCGATT GGCAGGCCGT GATGTCGACC AACCTCGACA GCATGTTCAA CGTCACCAAG 360
CAGGTGATCG AGGGCATGCT GGACAAGGGC TGGGGCCGGA TCATCAACAT CTCCTCGGTC 420
AACGGCGAGA AGGGCCAGTT CGGCCAGACC AACTACTCCG CCGCCAAGGC CGGCATGCAC 480
GGCTTCTCGA TGGCGCTGGC GCAGGAAGTG GCGGCCAAGG GCGTGACGGT GAACACCGTG 540
AGCCCGGGCT ACATCGCCAC GGACATGGTC AAGGCCATCC GCCAGGACGT GCTGGACAAG 600
ATCATCGCCA CCATTCCCAT CCGTCGCCTG GGTACGCCGG AGGAGATCGC CTCCATCGTC 660
GCCTGGCTGG CCGGCGAGGA GTCGGGCTTC ACCACCGGTG CCGACTTCAG CTGCAACGGC 720
GGCCTGCACA TGGGCTGA 738

(2) INFORMATION FOR SEQ ID NO. : 5:

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 536 amino acids

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 5:

Met Ser Gly Leu Asn Leu Pro Met Gln Ala Met Thr Lys Leu Gln Gly
5 10 15
Glu Tyr Leu Asn Glu Ala Thr Ala Leu Trp Asn Gln Thr Leu Gly Arg
20 25 30
Leu Gln Pro Asp Gly Ser Ala Gln Pro Ala Lys Leu Gly Asp Arg Arg
35 40 45
Phe Ser Ala Glu Asp Trp Ala Lys Asn Pro Ala Ala Ala Tyr Leu Ala
50 55 60
Gln Val Tyr Leu Leu Asn Ala Arg Thr Leu Met Gln Met Ala Glu Ser
65 70 75 80
Ile Glu Gly Asp Ala Lys Ala Lys Ala Arg Val Arg Phe Ala Val Gln
85 90 95
Gln Trp Ile Asp Ala Ala Ala Pro Ser Asn Phe Leu Ala Leu Asn Pro
100 105 110
Glu Ala Gln Arg Lys Ala Leu Glu Thr Lys Gly Glu Ser Ile Ser Gln
115 120 125
Gly Leu Gln Gln Leu Trp His Asp Ile Gln Gln Gly His Val Ser Gln
130 135 140
Thr Asp Glu Ser Val Phe Glu Val Gly Lys Asn Val Ala Thr Thr Glu
145 150 155 160
Gly Ala Val Val Tyr Glu Asn Asp Leu Phe Gln Leu Ile Glu Tyr Lys
165 170 175
Pro Leu Thr Pro Lys Val His Glu Lys Pro Met Leu Phe Val Pro Pro
180 185 190
Cys Ile Asn Lys Tyr Tyr Ile Leu Asp Leu Gln Pro Asp Asn Ser Leu
195 200 205
Ile Arg Tyr Thr Val Ala Gln Gly His Arg Val Phe Val Val Ser Trp
210 215 220

Arg 225	Asn	Pro	Asp	Ala	Ser 230	Val	Ala	Gly	Lys	Thr 235	Trp	Asp	Asp	Tyr	Val 240
Glu	Gln	Gly	Val	Ile 245	Arg	Ala	Ile	Arg	Val 250	Met	Gln	Gln	Ile	Thr 255	Gly
His	Glu	Lys	Val 260	Asn	Ala	Leu	Gly	Phe 265	Cys	Val	Gly	Gly	Thr 270	Ile	Leu
Ser	Thr	Ala 275	Leu	Ala	Val	Leu	Ala 280	Ala	Arg	Gly	Glu	Gln 285	Pro	Ala	Ala
Ser	Leu 290	Thr	Leu	Leu	Thr	Thr 295	Leu	Leu	Asp	Phe	Ser 300	Asn	Thr	Gly	Val
Leu 305	Asp	Leu	Phe	Ile	Asp 310	Glu	Ala	Gly	Val	Arg 315	Leu	Arg	Glu	Met	Thr 320
Ile	Gly	Glu	Lys	Ala 325	Pro	Asn	Gly	Pro	Gly 330	Leu	Leu	Asn	Gly	Lys 335	Glu
Leu	Ala	Thr	Thr 340	Phe	Ser	Phe	Leu	Arg 345	Pro	Asn	Asp	Leu	Val 350	Trp	Asn
Tyr	Val	Val 355	Gly	Asn	Tyr	Leu	Lys 360	Gly	Glu	Ala	Pro	Pro 365	Pro	Phe	Asp
Leu	Leu 370	Tyr	Trp	Asn	Ser	Asp 375	Ser	Thr	Asn	Met	Ala 380	Gly	Pro	Met	Phe
Cys 385	Trp	Tyr	Leu	Arg	Asn 390	Thr	Tyr	Leu	Glu	Asn 395	Lys	Leu	Arg	Val	Pro 400
Gly	Ala	Leu	Thr	Ile 405	Cys	Gly	Glu	Lys	Val 410	Asp	Leu	Ser	Arg	Ile 415	Glu
Ala	Pro	Val	Tyr 420	Phe	Tyr	Gly	Ser	Arg 425	Glu	Asp	His	Ile	Val 430	Pro	Trp
Glu	Ser	Ala 435	Tyr	Ala	Gly	Thr	Gln 440	Met	Leu	Ser	Gly	Pro 445	Lys	Arg	Tyr
Val	Leu 450	Gly	Ala	Ser	Gly	His 455	Ile	Ala	Gly	Val	Ile 460	Asn	Pro	Pro	Gln
Lys 465	Lys	Lys	Arg	Ser	Tyr 470	Trp	Thr	Asn	Glu	Gln 475	Leu	Asp	Gly	Asp	Phe 480

Asn Gln Trp Leu Glu Gly Ser Thr Glu His Pro Gly Ser Trp Trp Thr
485 490 495
Asp Trp Ser Asp Trp Leu Lys Gln His Ala Gly Lys Glu Ile Ala Ala
500 505 510
Pro Lys Thr Pro Gly Asn Lys Thr His Lys Pro Ile Glu Pro Ala Pro
515 520 525
Gly Arg Tyr Val Lys Gln Lys Ala
530 535 536

(2) INFORMATION FOR SEQ ID NO. : 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 392 amino acids

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 6 :

Met Thr Asp Ile Val Ile Val Ala Ala Ala Arg Thr Ala Val Gly Lys
5 10 15
Phe Gly Gly Thr Leu Ala Lys Thr Pro Ala Pro Glu Leu Gly Ala Val
20 25 30
Val Ile Lys Ala Leu Leu Glu Lys Thr Gly Val Lys Pro Asp Gln Ile
35 40 45
Gly Glu Val Ile Met Gly Gln Val Leu Ala Ala Gly Ala Gly Gln Asn
50 55 60
Pro Ala Arg Gln Ala Met Met Lys Ala Gly Ile Ala Lys Glu Thr Pro
65 70 75 80
Ala Leu Thr Ile Asn Ala Val Cys Gly Ser Gly Leu Lys Ala Val Met
85 90 95

Leu	Ala	Ala	Gln	Ala	Ile	Ala	Trp	Gly	Asp	Ser	Asp	Ile	Val	Ile	Ala
			100					105					110		
Gly	Gly	Gln	Glu	Asn	Met	Ser	Ala	Ser	Pro	His	Val	Leu	Met	Gly	Ser
		115					120					125			
Arg	Asp	Gly	Gln	Arg	Met	Gly	Asp	Trp	Lys	Met	Val	Asp	Thr	Met	Ile
	130					135					140				
Asn	Asp	Gly	Leu	Trp	Asp	Val	Tyr	Asn	Lys	Tyr	His	Met	Gly	Ile	Thr
145					150					155					160
Ala	Glu	Asn	Val	Ala	Lys	Glu	His	Asp	Ile	Ser	Arg	Asp	Gln	Gln	Asp
				165					170					175	
Ala	Leu	Ala	Leu	Ala	Ser	Gln	Gln	Lys	Ala	Thr	Ala	Ala	Gln	Glu	Ala
			180					185					190		
Gly	Arg	Phe	Lys	Asp	Glu	Ile	Val	Pro	Val	Ser	Ile	Pro	Gln	Arg	Lys
		195					200					205			
Gly	Asp	Pro	Val	Leu	Phe	Asp	Thr	Asp	Glu	Phe	Ile	Asn	Lys	Lys	Thr
	210					215					220				
Thr	Ala	Glu	Ala	Leu	Ala	Gly	Leu	Arg	Pro	Ala	Phe	Asp	Lys	Ala	Gly
225					230					235					240
Ser	Val	Thr	Ala	Gly	Asn	Ala	Ser	Gly	Ile	Asn	Asp	Gly	Ala	Ala	Ala
				245					250					255	
Val	Met	Val	Met	Ser	Ala	Ala	Lys	Ala	Lys	Glu	Leu	Gly	Leu	Thr	Pro
			260					265					270		
Met	Ala	Arg	Ile	Lys	Ser	Phe	Gly	Thr	Ser	Gly	Leu	Asp	Pro	Ala	Thr
		275					280					285			
Met	Gly	Met	Gly	Pro	Val	Pro	Ala	Ser	Arg	Lys	Ala	Leu	Glu	Arg	Ala
	290					295					300				
Gly	Trp	Gln	Val	Gly	Asp	Val	Asp	Leu	Phe	Glu	Leu	Asn	Glu	Ala	Phe
305					310					315					320
Ala	Ala	Gln	Ala	Cys	Ala	Val	Asn	Lys	Glu	Leu	Gly	Val	Asp	Pro	Ala
				325					330					335	
Lys	Val	Asn	Val	Asn	Gly	Gly	Ala	Ile	Ala	Ile	Gly	His	Pro	Ile	Gly
			340					345					350		

Ala	Ser	Gly	Cys	Arg	Val	Leu	Val	Thr	Leu	Leu	His	Glu	Met	Gln	Arg
		355					360					365			
Arg	Asp	Ala	Lys	Lys	Gly	Leu	Ala	Ala	Leu	Cys	Ile	Gly	Gly	Gly	Met
	370					375					380				
Gly	Val	Ser	Leu	Thr	Val	Glu	Arg								
385					390		392								

(2) INFORMATION FOR SEQ ID NO. : 7

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 245 amino acids

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 7 :

Met	Ala	Gln	Lys	Leu	Ala	Tyr	Val	Thr	Gly	Gly	Met	Gly	Gly	Ile	Gly
				5					10					15	
Thr	Ser	Met	Cys	Gln	Arg	Leu	His	Lys	Asp	Gly	Phe	Lys	Val	Ile	Ala
			20					25					30		
Gly	Cys	Gly	Pro	Ser	Arg	Asp	His	Gln	Lys	Trp	Ile	Asp	Glu	Gln	Ala
		35					40					45			
Ala	Leu	Gly	Tyr	Thr	Phe	Tyr	Ala	Ser	Val	Gly	Asn	Val	Ala	Asp	Trp
	50					55					60				
Asp	Ser	Thr	Val	Ala	Ala	Phe	Glu	Lys	Val	Lys	Ala	Glu	His	Gly	Thr
65					70					75					80
Val	Asp	Val	Leu	Val	Asn	Asn	Ala	Gly	Ile	Thr	Arg	Asp	Gly	Gln	Phe
				85					90					95	
Arg	Lys	Met	Ser	Lys	Ala	Asp	Trp	Gln	Ala	Val	Met	Ser	Thr	Asn	Leu
			100					105					110		

	Asp	Ser	Met	Phe	Asn	Val	Thr	Lys	Gln	Val	Ile	Glu	Gly	Met	Leu	Asp
			115					120					125			
	Lys	Gly	Trp	Gly	Arg	Ile	Ile	Asn	Ile	Ser	Ser	Val	Asn	Gly	Glu	Lys
		130					135					140				
5	Gly	Gln	Phe	Gly	Gln	Thr	Asn	Tyr	Ser	Ala	Ala	Lys	Ala	Gly	Met	His
	145					150					155					160
	Gly	Phe	Ser	Met	Ala	Leu	Ala	Gln	Glu	Val	Ala	Ala	Lys	Gly	Val	Thr
					165					170					175	
	Val	Asn	Thr	Val	Ser	Pro	Gly	Tyr	Ile	Ala	Thr	Asp	Met	Val	Lys	Ala
10				180					185					190		
	Ile	Arg	Gln	Asp	Val	Leu	Asp	Lys	Ile	Ile	Ala	Thr	Ile	Pro	Ile	Arg
			195					200					205			
	Arg	Leu	Gly	Thr	Pro	Glu	Glu	Ile	Ala	Ser	Ile	Val	Ala	Trp	Leu	Ala
		210					215					220				
15	Gly	Glu	Glu	Ser	Gly	Phe	Thr	Thr	Gly	Ala	Asp	Phe	Ser	Cys	Asn	Gly
	225					230					235					240
	Gly	Leu	His	Met	Gly											
					245											

20

(2) INFORMATION FOR SEQ ID NO. : 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 315 base pairs

(B) TYPE : nucleic acid

25 (C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULAR TYPE : promoter gene

(xi) SEQUENCE DESCRIPTION : SEQ ID NO. : 8:

30

ACACCGCGCC GAGCAAGGTG CCGTTGGGCG CCATGGCTTC GGCCACGGCC ATCATCAGCA 60

CCACGTAACA GCCATGCCAG AGCAACCAAG TACATAGCAA AAACCCGCAA TTACGCAGAA 120

TGACGTATTT CGTACAATGA AAACTGTTGT CATGATGCGG TAAGACACGA AGCCTACAAC 180

GCGATCCAGC AACGGTTTTC GTGAAAAAGT CCTCAGGAGA CGAGCGTGAC ACTGCAAATC 240

CCATTCCCGC ACTGCAACAG CTTGGCGACA ACGCCACGGC GCTGAGTGCC GCCATCTGGG 300

AACGTGCGCG CGATG 315

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00031

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: C 12 N 15/52,15/53,15/54,1/21 // (C 12 N 1/21; C 12 R 1:05,1:09)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: C 12 N 15/52,15/54,1/21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/19 747 A1 (IMPERIAL CHEMICAL INDUSTRIES PLC) 12 November 1992 (12.11.92), claims 1,3,5.	1
X	WO 95/05 472 A2 (MICHIGAN STATE UNIVERSITY) 23 February 1995 (23.02.95), claims 1,13,14.	1
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X	WO 93/02 194 A1 (IMPERIAL CHEMICAL INDUSTRIES PLC) 04 February 1993 (04.02.93), abstract.	1

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 May 1999 (04.05.99)	31 May 1999 (31.05.99)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer Wolf Telephone No. 1/53424/436

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00031

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